



Determination of Aflatoxin M1 Contamination and Integrity as well as Credibility

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Abstract

Aspergillums can produce and secrete directly aflatoxin M1. During the previous decade several papers pertaining to aflatoxin M1 have been published in different journals. Not mention of their more or less scientific aspects, they have fundamentally some problems in different features. In this paper we are going to have a bird's eye view on some articles published on this topic. It is suggested that complete research must be performed in order to find out the source of aflatoxin M1 contamination.

Keywords: Aflatoxin, Contamination, Fungus, Iran

Introduction

We noticed that in the past decade several papers pertaining to aflatoxin M1 were published. These papers have fundamentally some scientific and technical problems as follows:

Samples collecting methods were statistically inadequate. For example, in one study during the winter 2006 only 72 samples of the pasteurized milk packages were tested.

1) This means that during one month less than 25 samples were collected.

2) It is not clear how many supermarkets subjected in this study.

3) The collected samples belonged to one factory or more.

If we assume that each day a batch of milk is produced, share of every product batch is one sample, thus, the statistical aspect is wrong because of every batch of milk is not representative of a season.

4) It is not clear how long the shelf life of milks is.

5) It is not clear that contamination has been occurred in different stages of production and it is not clear the used samples in that study belong to one farms or more? It is possible that contamination has been occurred in one farm and other samples are being contaminated.

6) It is not clear the assay for the presence of aflatoxin M1 in samples milks carried out in one sea-

son or not. Finally, these researches do not meet with existing standards and confirmatory tests. In fact, these researches have not used the proper standard conditions.

Therefore, how we can prove the molecules resembling to aflatoxin M1 do not react with the ELISA kit as a false positive. However, in all of those studies, there are no external positive and negative controls. Generally, we believe that the ELISA kits without advanced confirmation tests are not valid for judgment. In addition, the authors do not talk about positive and negative controls samples, and possibility of secondary contamination has not considered.

Aspergillums can produce and secrete directly aflatoxin M1. Since, based on our observation, the mold contamination in cheese packaging in during the time and keeping in poor conditions can occur. In this regards, the only source of aflatoxin M1 is not aflatoxin B1 and under conditions, some strains of the fungus produce directly aflatoxin M1. For example, several articles have been published showing that Aspergillums are able to produce considerable amounts of aflatoxin M1 in suitable environment (1, 2). This means that any investigation about milk contamination by aflatoxin M1 regardless of the points mentioned may not be able to conclude that the milk has been contaminated. However, we should not ignore two basic assumptions:

1- Naturally, pasteurized milk is often contaminated with fungus spores can change to vegetative form and produce aflatoxin M1 after packaging.

2- Aflatoxin M1-like molecule may be present in milk or other dairy products that can give false positive reactions.

However, according to existence the defects in published articles lead us to have deeper investigation about researches which have been reported the existence of aflatoxin M1 contamination more than standard limit and have forced citizens to avoid dairy products and to pay attention subsequent indemnity. Recent research has shown that 50 ng /kg of aflatoxin M1 in Fisher mice after two years lead to hepatocarcinogenesis and adenocarcinogenesis (3). Thus we have logical reason to suspect that individuals who consume aflatoxin

M1 contaminated dairy products regularly, may develop liver and gastrointestinal cancer.

Elgebri et al., investigated the aflatoxin M1 contamination of milk and cheese samples in Africa. Their findings showed that 71.4 % of samples had been contaminated (30 to 3130 ng/liter). The important point in their study was that the aflatoxin M1 –free milk samples were contaminated with different concentrations of the aflatoxin M1. They were shown that the recovery of aflatoxin M1 compare to natural contamination was less (4).

Although these researchers, have carrying out the experimental and control contamination have shown the yield of aflatoxin M1 and accuracy of diagnostic method. However, the number of individuals who consume dairy products with contamination of 3130 ng/liter (6 and 60-fold more than American and European limitation respectively) was not mentioned. In addition, they were clarified as one of the main problems of published articles.

However, the study of resources in these cases showed that more than 40 Iranian papers related to amount of contamination levels of aflatoxin M1 in milk and other dairy products as well as various agricultural products has been published in Persian or English language. These articles can be divided in two groups: a) the articles which used ELISA technique. b) the articles which used HPLC methods.

For example, in 2007 a group of researchers examined the pasteurized and sterilized milk in stores Babol City in winter. They showed that the amount of contamination pasteurized milk was equals 230.5 and sterilized milk was equals 221.6 ng/liter (5). In 2008 another investigators studied the aflatoxin M1 in sterile milk via ELISA method and showed approximately 79.92% of samples had contaminated above the 50 ng/kg according to Europe Union Standards (6). They did not pay attention to practical solutions and concluded that most of the contamination of sterilized milk to aflatoxin M1 can be harmful to human health. In the same year, the amount of aflatoxin M1 contamination in milk production in Iran was studied by using competitive ELISA method. The results indicate that 70.7% of samples contaminated had

aflatoxin M1 more than European standard limit as well 26.9% of samples had aflatoxin M1 more than America standard limit (7).

Similarly, some investigators measured the amount of aflatoxin M1 contamination in raw milk, pasteurized milk and Ultra High temperature (UHT) milk were taken from stores of Esfahan City by means of ELISA kit and showed that 55.9% of samples have contamination over European standard limit (8).

In 2008, aflatoxin M1 in raw milk in Babol City was measured by means of ELISA method. 56.7% of the milks were infected to mycotoxin and contamination levels from 50 to 352 ng/liter are estimated (9). Other researchers, evaluated pasteurized milks by using ELISA kit in Mashhad City and demonstrated that 100% milks were contaminated to aflatoxin M1 and contamination 5.4% milks is more than European limitation (10).

In 2010 two investigators, separately published the results of their study on aflatoxin M1 contamination of milk and cheese in Tehran (11, 12). One of these investigators claimed that Iran national standard of aflatoxin M1 was 50 ng/liter and the other mentioned that Iran national standard of aflatoxin M1 was 200 ng/kg. It is not clear that which national reference approve these claims. In 2009 another investigator groups studied the amount of aflatoxin M1 in pasteurized milk consumed in Kerman City by means of HPLC with fluorescence detector and demonstrated that the amount of aflatoxin M1 contamination was below the American standard limit but 31 samples (44.7%) of the studied milk had contaminated over the European limit (13). These researchers express that there is no risk to public health, however due to high consumption of milk in children, aflatoxin M1 in this group of consumer to be considered a serious threat. They had not shown the average

amount of pasteurized milk consumption by Kermanian children. Moreover, in another study 319 milk samples of 15 dairy factories from across the country in during winter and summer were investigated by immunoaffinity HPLC column. The results revealed that the 54% of the samples were contaminated with aflatoxin M1 (14). The main research analysis results are shown in Table1. Although, it seems that selected tests have been in accordance with international standard but there is no reason that why aflatoxin M1 limit in American should be 0.5µg/kg and in European must be 0.05µg/kg (15, 16). In fact, the level selected 10-fold of limitation of the two regions has not been analyzed.

In the standard experimental tests of aflatoxin M1 contamination of milk (10 to 50 ng/kg) had shown that the RSDr \geq 30% and RSDR \geq 50%. Meanwhile, recycling level is noted 60 to 120 percent inoculate concentration. While, the results of the experimental tests with a more contamination concentration of 50 ng/kg of milk was showed RSDr \geq 20%, and RSDR \geq 30% and recycling level was noted 70 to 110 percent respectively (17). Therefore, an important question is that, why should there be 20% aflatoxin M1 be more recyclable in experimental tests? This fact indicates that probably there should be combination in milk that similar reaction is carried out with aflatoxin M1. But what is this component? It is not known that this component when appears in the milk. It need to careful and comprehensive more studies. Hence, based on the above idea the portable and rapid systems for detection of aflatoxin M1 in farm or livestock are designed and provide to increase the accuracy of diagnostic tools (18). In this case, the factories must be test the milk purchased in produced place and without delay.

Table 1: Summarized results of the researches on milk contamination to Aflatoxin M1 in different seasons

(ng/lit)				Origin	Region	Authors	Year
Winter	Autumn	Summer	Spring				
11- 20	-	20- 40 *	-	Milk (dairy industry)	Over the country	Taj Karimi et al.	2007
230/5	-	-	-	Pasteurized milk	Babol	Azizi et al.	2007
221/6	-	-	-	Sterilize milk			
-	-	-	13- 89	Pasteurized milk	Mashhad	Karimi et al.	2007
4- 352/3	-	-	-	Raw milk	Babol	Sefidgar et al.	2008
34- 211	24- 218	19/4- 93/6	87/4 -22/4	Sterilize milk	Tehran	Kamkar	2008
32- 879	32- 640	-	51- 914	Dry milk	Tehran	Kamkar	2008
-	13- 123	12- 152	-	Raw, pasteurized and UHT milk	Esfahan	Rahimi et al.	2009
2- 140	-	-	-	Pasteurized milk	Kerman	Pournourmoham-madi et al.	2009

* reported concentrations are according to ng/liter or kilogram.

Conclusion

Some materials or chemicals may be present in milk that may show a similar reaction in aflatoxin M1 kite measurement. However, each study that designed to measure the concentration of aflatoxin M1 should be considering the following cases.

1. Various chemical components of the environment and also food to animal feed contaminated with aflatoxin M1-producing fungal spores must be considered, because of the fungal spores may be change to vegetative form and produce this mycotoxin. Therefore, we must find a way to reduce population of fungi, particularly in the farms and livestock. Perhaps the use of gene deletion methods and also techniques based on antagonistic biological reactions is appropriate.

2. Various systems for detecting and measuring concentration of the mycotoxins is designed and developed, but for different reasons, none of the methods were not enough comprehensive and often based on existing facilities. Thus, the standard scientific principles are not considered. For these reasons, the studies have not only solved the problem but also concerns will be raised. Therefore, the results of these studies should be validated and offers appropriate solutions. This is notable that, why permissible limit of aflatoxin M1

in American was considered as 100 fold more than European limitation

3. The product in ELISA-based kit systems should be free of mycotoxin as negative control and unfortunately has not been considered in any of the tests. Also the amount of purified aflatoxin used in positive control is not mentioned. In addition, it is unclear, however the effects of preservatives in process of testing is unknown. Moreover,

the factories may produce standard mycotoxin which may contaminate with preservative. This can be affecting the results of the test.

4. In chromatography-based systems, especially HPLC, in no cases the characteristics of standard peak is compared with the peak of the sample tested.

5. It is suggested that complete research must be performed in order to find out the source and possible exact of aflatoxin M1 contamination.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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